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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR		ATTORNEY DOCKET NO.
09/058,323	04/09/98	HOUWEN	B	10690/101683

HM12/0724

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EXAMINER	
GABEL, G	
ART UNIT	**PAPER NUMBER**
1641	18

DATE MAILED: 07/24/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR / PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
09/058323	4/9/98	Houwen	

EXAMINER
G. Gabel.

ART UNIT	PAPER
1641	17

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Commissioner of Patents and Trademarks

Advisory Action	Application No. 09/058,323	Applicant(s) HOUWEN ET AL.	
	Examiner Gailene R. Gabel	Art Unit 1641	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 23 April 2001 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE. Therefore, further action by the applicant is required to avoid abandonment of this application. A proper reply to a final rejection under 37 CFR 1.113 may only be either: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114.

PERIOD FOR REPLY [check either a) or b)]

a) ☒ The period for reply expires 5 months from the mailing date of the final rejection.

b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.
ONLY CHECK THIS BOX WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

1. ☒ A Notice of Appeal was filed on 21 May 2001. Appellant's Brief must be filed within the period set forth in 37 CFR 1.192(a), or any extension thereof (37 CFR 1.191(d)), to avoid dismissal of the appeal.

2. ☐ The proposed amendment(s) will not be entered because:

   (a) ☐ they raise new issues that would require further consideration and/or search (see NOTE below);

   (b) ☐ they raise the issue of new matter (see Note below);

   (c) ☐ they are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or

   (d) ☐ they present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: _____.

3. ☐ Applicant's reply has overcome the following rejection(s): _____.

4. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).

5. ☒ The a)☐ affidavit, b)☐ exhibit, or c)☒ request for reconsideration has been considered but does NOT place the application in condition for allowance because: See Continuation Sheet.

6. ☐ The affidavit or exhibit will NOT be considered because it is not directed SOLELY to issues which were newly raised by the Examiner in the final rejection.

7. ☒ For purposes of Appeal, the proposed amendment(s) a)☐ will not be entered or b)☒ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.

   The status of the claim(s) is (or will be) as follows:

   Claim(s) allowed: NONE.

   Claim(s) objected to: NONE.

   Claim(s) rejected: 1-13.

   Claim(s) withdrawn from consideration: _____.

8. ☐ The proposed drawing correction filed on _____ is a)☐ approved or b)☐ disapproved by the Examiner.

9. ☐ Note the attached Information Disclosure Statement(s)(PTO-1449) Paper No(s). _____.

10. ☐ Other:

Continuation of 5. does NOT place the application in condition for allowance because: claims 1-13 fail to obviate the pending obviousness rejection over Loken in view of Kim and Inami and Applicant's argument on the Examiner's various reasons for combining the same references, to overcome the rejection is not persuasive. .

SUPPLEMENTAL ADVISORY ACTION

Amendment Entry

1. Applicants' amendment and response filed 4/23/01 in Paper No. 13 is acknowledged and has been entered. Claims 4 and 13 have been amended. Currently, claims 1-13 are pending and under examination.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Claim 13, as amended, is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 13 is vague and indefinite in reciting "the osmolarity of the leucocytes" because it appears that Applicant intends to make reference to a reagent fluid. Refer to pages 12-13.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103© and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

3. Claims 1-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Loken et al. (US 5,047,321) in view of Kim et al. (US 5,559,037) and Inami et al. (US 5,298,426) for reason of record in Paper No. 12.

Response to Arguments

4. A) Applicant seeks explanation of the shift of Examiner's position from *Inami in view of Loken* and *Kim in view Loken* in Paper No. 2 to *Loken in view of Kim and Inami* in Paper No. 12.

Contrary to Applicant's contention in paragraphs 2 and 3 in page 7 of Paper No. 13, however, the §103 rejection was over Kim in view of Loken (not over Loken in view of Kim) and over Inami in view of Loken (not over Loken in view of Inami) in Paper No. 2.

In response, Applicant's argument in Paper No. 9 filed 4/10/00 was rendered persuasive in having argued that neither Kim nor Inami teaches simultaneous analysis

of a sample using 1) erythroblast nucleotide dye staining and 2) leucocyte cell surface marker fluorescent labeling which are critical elements recited in claim 1, and therefore the rejection was withdrawn. Alternatively, Loken et al. was found to have provided flow cytometric analysis of nucleated cell populations teaching the use of 1) nucleotide dye staining of nuclear material in specific nucleated cell populations and 2) leucocyte cell surface marker fluorescent labeling and was, therefore, rendered as a stronger primary reference in establishing a prima facie case of obviousness in a §103 rejection. The withdrawal of the rejection and subsequent recombination of the references in Paper No. 10 was appropriately presented to Applicant as a Non-Final Office Action responsive to Applicant's previous amendment and persuasive argument. This subsequent rejection of claims 1-13 under 35 U.S.C. 103(a) as being unpatentable over Loken et al. (US 5,047,321) in view of Kim et al. (US 5,559,037) and Inami et al. (US 5,298,426) has since been maintained.

To reiterate, Loken uses flow cytometry to simultaneously analyze fluorescence intensity and light scatter between nucleated cell populations or lineages in a whole blood or bone marrow sample including erythroblasts and leucocytes. Loken uses 1) nucleotide fluorescent dyes that can permeate cell membrane to stain certain cell nuclei and 2) at least one fluorescent labeled antibody to label leucocyte cell surface antigens. Kim teaches flow cytometric analysis of stained erythroblasts and leucocytes but incorporating a diluent to lyse the erythroblasts in order to expose their nuclei for staining while preserving the integrity and shape of leucocytes. Kim constructs three-dimensional plots of signals of fluorescence and scattered light to differentiate between

erythroblasts and leucocytes. Inami uses flow cytometric analysis in differentiating between stained erythroblasts and stained leucocytes but incorporating 1) hypotonic fluorescent dye solution to enable diffusion of nucleotide fluorescent dye into erythroblasts in a buffer for maintaining the pH in the acidic range, and 2) a buffer that neutralizes the acidic pH in the mixture and an osmolarity adjusting agent that adjusts osmolarity to retain the shape and integrity of leucocytes.

It would have been obvious to one of ordinary skill in the art at the time of the invention to combine teachings of Kim and Inami in increasing cell membrane permeability of erythroblasts in differentiating between two "stained" nucleated cell populations in a blood sample: nRBC and leucocytes, into the method of Loken that uses 1) nucleic acid dyes to stain nuclear material in specific cell lineages, i.e. erythroblasts and 2) fluorescent labeled antibodies to label leucocyte cell surface markers in differentiating between nucleated cell populations, being that 1) all three references share the same purpose in the use of flow cytometry to define cellular populations based on nuclear content differentiation, 2) all three references recognize the importance of accuracy in differentiating between two nucleated cell populations in a blood sample for diagnostic purposes, i.e. leukemia, anemia, lymphoma, leukopenia, etc., 3) all three references taught and suggest improvements in acquiring increased levels of accuracy and ease in differentiation of nucleated cell populations in order to provide precise diagnosis of these diseases (Inami: column 1, lines 26-31, Kim: column 1, lines 14-34, Loken et al., column 1). All three references recognize that effective staining or labeling between the nucleated cell populations is key and requisite to

providing accurate differentiation. Loken identified nuclear dyes that can penetrate cell membrane. Inami and Kim specifically taught erythroblast cell membrane permeabilization methods to effect penetration of stain through cell membrane into intracellular environment in order to effect better staining of nuclear material. Inami specifically taught manipulation of pH and osmolarity of buffers in the system to protect integrity of other cell populations, i.e. the leucocyte population in the sample. It follows that one of ordinary skill in the art at the time of the instant invention would have been motivated to combine the teachings of Kim and Inami with the method of Loken in differentiating between nucleated hematopoietic cell populations because effective staining technique of nuclear material in different nucleated cell populations, i.e. erythroblastic and leucocyte populations, enables better differentiation therebetween; thereby, providing for accurate discrimination, ease, and timely diagnosis of disease in simultaneous nucleated cell differentiation methods.

In response to Applicant's argument that the same or "identical" line of reasoning was maintained between the former and latter combination of references, the Examiner sets forth as aforementioned that all three references, albeit recombined, teach the same analogous art and share the same motivation and purpose in their study of cellular differentiation based on nuclear staining and/or cell surface marker labeling and, therefore, it should not be unusual that the same or identical line of reasoning is taught or suggested if recombined - it would otherwise be odd to deviate from them.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections

are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

5. Applicant's arguments filed 4/23/01have been fully considered but they are not persuasive. Accordingly, no claims are allowed.

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gail Gabel whose telephone number is (703) 305-0807. The examiner can normally be reached on Monday to Thursday from 7:00 AM to 4:30 PM. The examiner can also be reached on alternate Fridays from 7:00 AM to 3:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le, can be reached on (703) 308-4027. The fax phone number for the organization where this application or proceeding is assigned is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Gail Gabel
Patent Examiner
Group 1641

LONG V. LE
SUPERVISORY PATENT EXAMINER
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07/18/01